REMARKS

Claims 1-3, 6-13, and 15 and 16 have been cancelled and new claims 17-24 have been added. No new subject matter is believed to be added. The narrowed scope of claim 17 is supported by cancelled claims 1 and 3 and Example 8. Support for claims 18 and 19 can be found in Examples 5 and 6. Support for claim 24 can be found for example on pages 8 and 36 of the application and in Figure 8.

The title, abstract and cross reference have been amended.

A replacement sequence listing is attached as a separate document with this response to comply with, in particular, 37 C.F.R. § 1.821(a)-(d) as requested by Examiner in the Office Action. No new matter has been added. Replacement Figure 9 and Replacement Appendix A, which now include sequence identifiers, are attached to this response.

The method of the claimed invention describes how to assemble a chimeric Type IIG restriction endonuclease and determine whether it has activity. The methylase domain is highly conserved in the Type IIG family of enzymes. The most highly conserved motifs in the conserved methylase domain are motifs I and IV which are now specifically claimed in claim 17 as the joining site for two different DNA fragments. The screening method for determining enzyme activity is described in detail in the specification and requires only surveying transformed host cell clones for a blue color. This provides a rapid simple

detection method for identifying chimeric enzymes formed by the claimed method using any member of the Type IIG group.

The Examiner has objected to the now cancelled claims in paragraphs 8-26 of the office action. The Examiner is thanked for the constructive comments. While Applicants traverse the objections, the claims have been cancelled and new claims have been added noting these comments.

Applicants request that the Examiner note that Motifs I and IV in claim 17 are defined by a consensus sequence.

Motif I in the methylase domain is well defined by the consensus sequence I/VLD/EPSCGXGXF/LL and Motif IV is defined by the consensus sequence F/YDXIIGNPPY as shown in Figure 9 in the Application.

The Examiner has objected to a Type IIG restriction endonuclease having a functionally inactive methylase domain. However, the cleavage and methylase domains are separate distinct functions of the fusion protein. Applicants have demonstrated that one function can be inactivated without destroying the other function. In Example 5, Type IIG restriction endonuclease cleavage activity is inactivated while maintaining methylase activity ($R^{+}M^{+}S^{+}$). Applicants have also subsequently created $R^{+}M^{+}S^{-}$ for a Type IIG restriction endonuclease that lacks methylase activity but has restriction endonuclease activity.

Therefore it is proper to claim in dependent form that the chimeric Type IIG restriction endonuclease has methylase activity.

The Examiner has rejected claims 1-3, 6-13 and 15-16 as failing to comply with the written description requirement on the basis that the nucleic acids encoding the domains of the Type IIG restriction endonuclease lack structure even where Figure 9 shows the consensus sequences identifying each motif. Moreover, the Examiner states that the Type IIG restriction endonucleases as a group are only loosely defined.

The Examiner has queried the inclusion of BpmI identified as a Type IIf endonuclease. Applicants used this classification in 2002 (when the parent application was filed) where "f" was used to denote "fusion". In 2003, Richard Roberts published a revised nomenclature for restriction endonucleases (*Nucleic Acids Research* 31:1805-1812 (2003)), attached hereto. In this nomenclature, BpmI was redefined and properly included in the Type IIG category because it was a single polypeptide having cleavage and methylase activities where the methylase shares the conserved motifs that characterize the Type IIG family.

The Type IIG family, although relatively modest in size, now has in excess of 20 different members. It has been speculated that this family of enzymes evolved from a single source because with the exception of this family, it is very unusual to find any sequence in common between proteins with restriction endonuclease activity.

Type IIG restriction endonucleases have the following characteristics:

(i) They contain a cleavage domain, a methylase domain and a specificity domain (TRD, also referred to as target recognition domain) to form a single polypeptide.

(ii) They cleave substrate at an approximately fixed distance outside the DNA recognition sequences.

(iii) A subset of Type IIG restriction endonucleases have a gamma type methylase* which is defined by motifs ordered as follows: X, I, II, III, IV, V, VI, VII and VIII, with the specificity domain (TRD) at the C-terminal end after Motif VIII as described in Figure 9 and in the Malone et al. reference (copy attached).

* It is generally known in the art that an alpha methylase and a beta methylase have motifs that are organized differently with respect to the specificity domain (TRD) than the gamma type methylase (see for example Figure 1A-1C in Malone et al).

The Examiner has rejected the claims on the basis of scope and enablement. However, the working examples provide detailed descriptions of how to make several different chimeric Type IIG restriction endonucleases. Example 1 provides a description of techniques for forming a fusion protein. Example 2 shows how to obtain a DNA sequence of a Type IIG restriction endonuclease. Examples 3, 5, 6 and 7 describe how to make deletion mutants of several Type IIG endonucleases for use as

DNA segments. Examples 2 and 8 provide detailed descriptions of how to form a chimeric Type IIG restriction endonuclease from the DNA segments including the use of linkers of varying sizes to enhance the activity of the chimeric restriction endonucleases and the use of the *in vivo* SOS induction assay to rapidly screen for restriction endonuclease activity of the chimeric enzymes.

The Examiner states that enablement is not precluded by the necessity of routine screening. Applicants respectfully assert that the claimed method meets this test of enablement.

CONCLUSION

For the reasons set forth above, Applicants respectfully submit that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants petition for a three-month extension of time and authorize that the extension fee of $525 be charged to Deposit Account No. 1-0740. Please charge any deficiencies to Deposit Account No. 14-0740.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

Date: January 9, 2008

Harriet M. Strimpel D.Phil.
(Reg. No.: 37,008)
Attorney for Applicant
240 Country Road
Ipswich, Massachusetts 01938
(978) 380-7373

Customer No.: 28986

Replacement Figure 9